CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment comprising

(a) a first nucleic acid subfragment encoding apartokinase which is substantially insensitive to inhibition by lysine; and

(b) a second nucleic acid subfragment encoding dihydrodipicolinic acid synthase which is substantially insensitive to inhibition by lysine.

2. The nucleic acid fragment of Claim 1, wherein either:

(a) the first nucleic acid subfragment comprises a nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1: encoding *E. coli* AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(1) the amino acid at position 318 is an amino acid other than threonine, or

(2) the amino acid at position 352 is an amino acid other than methionine; or

(b) the second nucleic acid subfragment is derived from bacteria.

3. An isolated nucleic acid fragment comprising a nucleic acid subfragment encoding lysine ketoglutarate reductase.

4. The nucleic acid fragment of Claim 1 further comprising:

(c) a third nucleic acid subfragment encoding part or all of lysine ketoglutarate reductase.

5. A nucleic acid fragment comprising

(a) a first chimeric gene wherein a nucleic acid fragment encoding apartokinase which is substantially insensitive to inhibition by lysine is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence; and

(b) a second chimeric gene wherein a nucleic acid fragment encoding dihydrodipicolinic acid synthase which is substantially insensitive to inhibition by lysine is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence.

6. The nucleic acid fragment of Claim 5 comprising a third chimeric gene wherein a nucleic acid fragment encoding part or all of lysine ketoglutarate reductase is operably linked in the sense or antisense orientation to a seed-specific regulatory sequence.

7. An isolated nucleic acid fragment comprising at least one nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1 encoding

E. coli AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(a) the amino acid at position 318 is an amino acid other than threonine, or

(b) the amino acid at position 352 is an amino acid other than methionine.

8. A chimeric gene wherein the nucleic acid fragment of Claim 7 is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence.

9. A plant comprising in its genome the nucleic acid fragment of Claim 5 or Claim 6.

10. A plant comprising in its genome the chimeric gene of Claim 8.

11. A seed obtained from the plant of Claim 9 comprising in its genome the nucleic acid fragment of Claim 5 or Claim 6, or from the plant of Claim 10 comprising in its genome the chimeric gene of Claim 8.

12. A method for increasing the threonine content of the seeds of plants comprising:

(a) transforming plant cells with the chimeric gene of Claim 8;

(b) growing fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds; and

(c) selecting from the progeny seed of step (b) for those seeds containing increased levels of threonine.

13. A method for increasing the lysine content of the seeds of plants comprising:

(a) transforming plant cells with the nucleic acid fragment of Claims 5;

(b) growing fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds; and

(c) selecting from the progeny seed of step (b) those seeds containing increased levels of lysine.

14. A plant produced by the method of Claim 12, wherein the plant is capable of transmitting said chimeric gene to a progeny plant and wherein the progeny plant has the ability to produce levels of free threonine at least two times greater than the free threonine levels of untransformed plants.

15. A plant produced by the method of Claim 13, wherein the plant is capable of transmitting said nucleic acid fragment to a progeny plant and wherein

the progeny plant has the ability to produce levels of free lysine at least two times greater than free lysine levels of plants not containing the nucleic acid fragment.

16. A plant comprising in its genome the first, second and third chimeric genes of Claim 6.

17. An isolated nucleic acid fragment comprising

(a) a first nucleic acid subfragment encoding apartokinase which is substantially insensitive to inhibition by lysine and

(b) a second nucleic acid subfragment encoding dihydrodipicolinic acid synthase which is substantially insensitive to inhibition by lysine and

(c) a third nucleic acid subfragment encoding a lysine-rich protein wherein the weight percent lysine is at least 15%.

18. The nucleic acid fragment of Claim 17, wherein either:

(a) the first nucleic acid subfragment comprises a nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1: encoding *E. coli* AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(1) the amino acid at position 318 is an amino acid other than threonine, or

(2) the amino acid at position 352 is an amino acid other than methionine; or

(b) the second nucleic acid subfragment is derived from bacteria; or

(c) the third nucleic acid subfragment encodes a lysine-rich protein wherein the weight percent lysine is at least 15%.

19. The nucleic acid fragment of Claim 17, wherein either:

(a) the first nucleic acid subfragment comprises a nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1: encoding *E. coli* AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(1) the amino acid at position 318 is an amino acid other than threonine, or

(2) the amino acid at position 352 is an amino acid other than methionine; or

(b) the second nucleic acid subfragment is derived from bacteria; or

(c) the third nucleic acid subfragment comprises a nucleic acid sequence encoding a lysine-rich protein comprising n heptad units (d e f g a b c), each heptad being either the same or different, wherein:

n is at least 4;

a and d are independently selected from the group consisting of Met, Leu, Val, Ile and Thr;

e and g are independently selected from the group consisting of the acid/base pairs Glu/Lys, Lys/Glu, Arg/Glu, Arg/Asp, Lys/Asp, Glu/Arg, Asp/Arg and Asp/Lys; and

b, c and f are independently any amino acids except Gly or Pro and at least two amino acids of b, c and f in each heptad are selected from the group consisting of Glu, Lys, Asp, Arg, His, Thr, Ser, Asn, Ala, Gln and Cys.

20. The nucleic acid fragment of Claim 17, wherein:

(a) the first nucleic acid subfragment comprises a nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1: encoding *E. coli* AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(1) the amino acid at position 318 is an amino acid other than threonine, or

(2) the amino acid at position 352 is an amino acid other than methionine; or

(b) the second nucleic acid subfragment is derived from bacteria; or

(c) the third nucleic acid subfragment comprises a nucleic acid sequence encoding a lysine-rich protein having the amino acid sequence $(MEEKLKA)_6(MEEKMKA)_2$.

21. A nucleic acid fragment comprising:

(a) a first chimeric gene wherein a nucleic acid fragment encoding apartokinase which is substantially insensitive to inhibition by lysine is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence and

(b) a second chimeric gene wherein a nucleic acid fragment encoding dihydrodipicolinic acid synthase which is substantially insensitive to inhibition by lysine is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence.

(c) a third chimeric gene wherein a nucleic acid fragment encoding a lysine-rich protein wherein the weight percent lysine is at least 15% is operably linked to a seed-specific regulatory sequence.

22. An isolated nucleic acid fragment comprising:

(a) a first chimeric gene wherein a nucleic acid fragment comprising a nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1: encoding *E. coli* AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(1) the amino acid at position 318 is an amino acid other than threonine, or

(2) the amino acid at position 352 is an amino acid other than methionine is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence and

(b) a second chimeric gene wherein a nucleic acid fragment derived from a bacteria encoding dihydrodipicolinic acid synthase is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence and

(c) a third chimeric gene wherein a nucleic acid fragment encoding a lysine-rich protein wherein the weight percent lysine is at least 15% is operably linked to a seed-specific regulatory sequence.

23. An isolated nucleic acid fragment comprising

(a) a first chimeric gene wherein a nucleic acid fragment comprising a nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1: encoding *E. coli* AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(1) the amino acid at position 318 is an amino acid other than threonine, or

(2) the amino acid at position 352 is an amino acid other than methionine is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence and

(b) a second chimeric gene wherein a nucleic acid fragment derived from a bacteria encoding dihydrodipicolinic acid synthase is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence and

(c) a third chimeric gene wherein a nucleic acid fragment encoding a lysine-rich protein comprising n heptad units (d e f g a b c), each heptad being either the same or different, wherein:

n is at least 4;

a and d are independently selected from the group consisting of Met, Leu, Val, Ile and Thr;

e and g are independently selected from the group consisting of the acid/base pairs Glu/Lys, Lys/Glu, Arg/Glu, Arg/Asp, Lys/Asp, Glu/Arg, Asp/Arg and Asp/Lys; and

b, c and f are independently any amino acids except Gly or Pro and at least two amino acids of b, c and f in each heptad are selected from the group consisting of Glu, Lys, Asp, Arg, His, Thr, Ser, Asn, Ala, Gln and Cys,

said nucleic acid fragment is operably linked to a seed-specific regulatory sequence.

24. An isolated nucleic acid fragment comprising

(a) a first chimeric gene wherein a nucleic acid fragment comprising a nucleotide sequence essentially similar to the sequence shown in SEQ ID NO:1: encoding *E. coli* AKIII, said nucleic acid fragment encoding a lysine-insensitive variant of *E. coli* AKIII and further characterized in that at least one of the following conditions is met:

(1) the amino acid at position 318 is an amino acid other than threonine, or

(2) the amino acid at position 352 is an amino acid other than methionine is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence and

(b) a second chimeric gene wherein a nucleic acid fragment derived from a bacteria encoding dihydrodipicolinic acid synthase is operably linked to a plant chloroplast transit sequence and to a seed-specific regulatory sequence and

(c) a third chimeric gene wherein a nucleic acid fragment encoding a lysine-rich protein having the amino acid sequence $(MEEKLKA)_6(MEEKMKA)_2$ is operably linked to a seed-specific regulatory sequence.

25. A plant comprising in its genome a nucleic acid fragment selected from the group consisting of the fragment of Claim 21, the fragment of Claim 22, the fragment of Claim 23 and the fragment of Claim 24.

26. A plant comprising in its genome each of the chimeric genes of Claims 21 or Claim 22 or Claim 23 or Claim 24.

27. A seed obtained from the plant of Claim 25 comprising in its genome a nucleic acid fragment selected from the group consisting of the fragment of Claim 21, the fragment of Claim 22, the fragment of Claim 23 and the fragment of Claim 24 or from the plant of Claim 26.

28. A method for increasing the lysine content of the seeds of plants comprising:

(a) transforming plant cells with a nucleic acid fragment selected from the group consisting of the fragment of Claim 21, the fragment of Claim 22, the fragment of Claim 23 and the fragment of Claim 24;

(b) growing fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds; and

(c) selecting from the progeny seed of step (b) those seeds containing increased levels of lysine.

29. A transformed plant wherein the seeds of the plant accumulate lysine at a level at least ten percent higher than do seeds of an untransformed plant.

30. A transformed plant, as described by Claim 29, wherein the seeds of the plant accumulate lysine at a level from ten percent to four-fold higher than do seeds of an untransformed plant.

31. A transformed rapeseed wherein the seeds of the plant accumulate lysine to a level between ten percent and one hundred percent higher than do seeds of an untransformed plant.

32. A transformed soybean plant wherein the seeds of the plant accumulate lysine to a level between ten percent and four-fold higher than do seeds of an untransformed plant.

33. The nucleic acid fragment of Claim 5 wherein the seed-specific regulatory sequence is a monocot embyro-specific promoter.

34. A monocot plant comprising in its genome the nucleic acid fragment of Claim 33.

35. A seed obtained from the plant of Claim 34 and comprising in its genome the nucleic acid fragment of Claim 33.

36. A method for increasing the lysine content of the seeds of monocot plants comprising:

(a) transforming plant cells with the nucleic acid fragment of Claim 33;

(b) growing fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds; and

(c) selecting from the progeny seed of step (b) those seeds containing increased levels of lysine.

37. A plant produced by the method of Claim 36, wherein the plant is capable of transmitting said nucleic acid fragment to a progeny plant and wherein the progeny plant has the ability to produce levels of free lysine at least five times greater than free lysine levels of plants not containing the nucleic acid fragment.

38. A transformed corn plant wherein the seeds of the plant accumulate lysine to a level between ten percent and one hundred thirty percent higher than do seeds of an untransformed plant.

39. A method for increasing the lysine content and reducing the accumulation of lysine breakdown products of the seeds of plants comprising:

(a) transforming plant cells with the nucleic acid fragment of Claim 6;

(b) growing fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds; and

(c) selecting from the progeny seed of step (b) those seeds containing increased levels of lysine and reduced levels of lysine breakdown products.

40. A method for increasing the lysine content and reducing the accumulation of lysine breakdown products of the seeds of plants comprising:

(a) transforming plant cells with the nucleic acid fragment of Claim 5;

(b) growing fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds;

(c) selecting from the progeny seed of step (b) those seeds containing increased levels of lysine; and lysine breakdown products and

(d) introducing a mutation in the gene encoding lysine ketoglutarate reductase which reduces the enzyme activity and reduces accumulation of lysine breakdown products.